

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Currently amended) A method of preparing a polynucleotide having a target sequence from a plurality of oligonucleotides, said method comprising:

(a) coupling said oligonucleotides by ligating with a ligase or ribozyme to form a plurality of coupled oligonucleotides, wherein each of said coupled oligonucleotides represents a region of sequence of said polynucleotide and shares at least one terminal region of sequence with at least one other coupled oligonucleotide; and

(b) assembling said polynucleotide by extension of said coupled oligonucleotides.

2. (Canceled)

3. (Currently amended) The method of claim [2] 1 wherein said ligase is T4 RNA ligase.

4. (Original) The method of claim 1 wherein at least one of said oligonucleotides of said coupled oligonucleotides is attached to solid support prior to coupling.

5. (Original) The method of claim 1 wherein said coupled oligonucleotides are attached to solid support.

6. (Original) The method of claim 1 wherein each of said coupled oligonucleotides is amplified prior to assembling said polynucleotide.

7. (Original) The method of claim 1 wherein at least one of said oligonucleotides of said coupled oligonucleotides is blocked at one end prior to coupling.

8. (Original) The method of claim 1 wherein said coupled oligonucleotides comprise pairs of oligonucleotides.

9. (Original) The method of claim 1 wherein said extension is carried out using overlap PCR.

10. (Canceled)

11. (Currently amended) The method according to claim 1, wherein:
the 3' end of each of said oligonucleotides is blocked, except for the oligonucleotide comprising the 5' terminus of said polynucleotide, with a blocking group to form a plurality of blocked oligonucleotides;
the 5' end of each of said blocked oligonucleotides is coupled with the 3' end of a further oligonucleotide of said plurality of oligonucleotides by ligating with a ligase or ribozyme to form a plurality of coupled oligonucleotides and wherein said further oligonucleotide comprises a portion of said polynucleotide immediately 5' to the sequence of said blocked oligonucleotide.

12. (Original) The method of claim 11 wherein said polynucleotide is DNA, RNA, or DNA/RNA hybrid.

13. (Original) The method of claim 11 wherein said oligonucleotides comprise from about 10 to about 200 nucleotides.

14. (Original) The method of claim 11 wherein said blocking group comprises solid support.

15. (Original) The method of claim 14 wherein said solid support is selected from the group consisting of agarose, polyacrylamide, magnetic beads, polystyrene, polyacrylate, controlled-pore glass, hydroxyethylmethacrylate, polyamide, polyethylene, polyethyleneoxy, and polyethyleneoxy/polystyrene copolymer.

16. (Previously presented) The method of claim 11 wherein said blocking group is biotin-dideoxyuridine triphosphate.

17. (Canceled)

18. (Currently amended) The method of claim [17] 11 wherein said ligase is T4 RNA ligase.

19. (Currently amended) The method of claim [17] 11 wherein said coupling comprises the steps of contacting said blocked oligonucleotide with ligase and cosubstrate to form activated oligonucleotide, washing said activated oligonucleotide to form washed oligonucleotide, and contacting said washed oligonucleotide with said further oligonucleotide and ligase.

20. (Original) The method of claim 11 wherein said coupled oligonucleotides are amplified prior to assembling said polynucleotide.

21. (Original) The method of claim 11 wherein said extension is carried out using overlap PCR.

22-56. (Canceled)